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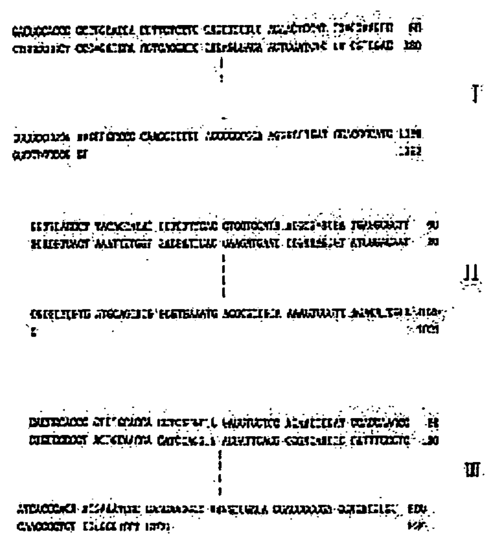
(71)Applicant : CANON INC
MARINE BIOTECHNOL INST CO LTD

(72)Inventor : YANO TETSUYA
NOMOTO TAKESHI
IMAMURA TAKESHI
HAMADA TORU

(54) NEW NUCLEIC ACID FRAGMENT AND DETECTION OF MICROORGANISM BY USING THE SAME

(57)Abstract:



PROBLEM TO BE SOLVED: To obtain a new nucleic acid fragment comprising a gyrB gene having a specific base sequence, and capable of rapidly, readily and specifically detecting and counting specified microorganisms capable of degrading trichloroethylene or the like and useful for purification of polluted environment, in good sensitivity.

SOLUTION: This fragment is the new nucleic acid fragment comprising a gyrB gene having a base sequence selected from formulas I to III, and useful for rapidly, readily and specifically detecting and counting microorganisms such as Vibrio sp. KB1 strain (FERM P-14643), JM1 strain (FERM BP-5352) and Pseudomonas cepacia KK01 strain (FERM BP-4235), capable of degrading trichloroethylene or the like and useful for purification of polluted environment in good sensitivity. The nucleic acid fragment is obtained by comparing base sequences of gyrB genes of various known Gram-negative bacteria and amplifying each nucleic acid obtained from the before microorganisms by using a primer set from a common sequence region obtained by the comparison, by a PCR.

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* NOTICES *

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1.This document has been translated by computer. So the translation may not reflect the original precisely.
2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

CLAIMS

[Claim(s)]
[Claim 1] The array number of an array table: The gyrB gene characterized by having the base sequence of either 1-6.
[Claim 2] Vibrio One share (FERM P-14643) of sp.KB, One share (FERM BP-5352) of JM, Pseudomonas cepacia KK01 share (FERM BP-4235), One share (FERM P-14726) of Pseudomonassp.TL, Alcaligenes Two shares (FERM P-14642) of sp.TL, and Pseudomonas alcaligenes As a candidate for detection chosen from two shares (FERM BP-5354) of KB(s) It is the nucleic-acid fragment which has the base sequence of ** as the probe for detecting a specific base sequence by the hybridization method in the gyrB gene of ** 1 strain. a) All the base sequences or the complementary array of those of the gyrB gene made applicable to detection, As opposed to all these base sequences a or the partial array b b) -- the gyrB gene made applicable [that was chosen from these all base sequences / this] to detection -- the partial array of a proper, or the complementary array and c -- The nucleic-acid fragment characterized by having the variation array acquired by performing insertion, the deletion, or the permutation of one or more bases within limits which do not spoil the function as a probe for gyrB gene detection, or its complementary array.
[Claim 3] The strain as said candidate for detection is Vibrio. Nucleic-acid fragment according to claim 2 with which it is one share (FERM P-14643) of sp.KB, and the gyrB gene has the base sequence of array number:1.
[Claim 4] The nucleic-acid fragment according to claim 2 with which the strain as said candidate for detection is one share (FERM BP-5352) of JM, and the gyrB gene has the base sequence of array number:2.
[Claim 5] The strain as said candidate for detection is Pseudomonas. cepacia Nucleic-acid fragment according to claim 2 with which it is KK01 share (FERM BP-4,235) and the gyrB gene has the base sequence of array number:3.
[Claim 6] The strain as said candidate for detection is Pseudomonas. Nucleic-acid fragment according to claim 2 with which it is one share (FERM P-14726) of sp.TL, and the gyrB gene has the base sequence of array number:4.
[Claim 7] The strain as said candidate for detection is Alcaligenes. Nucleic-acid fragment according to claim 2 with which it is two shares (FERM P-14642) of sp.TL, and the gyrB gene has the base sequence of array number:5.
[Claim 8] The strain as said candidate for detection is Pseudomonas. alcaligenes Nucleic-acid fragment according to claim 2 with which it is two shares (FERM BP-5354) of KB(s), and the gyrB gene has the base sequence of array number:6.
[Claim 9] The nucleic-acid fragment according to claim 2 to 8 which is ten or more bases and has the die length to the same number of bases as said gyrB gene.
[Claim 10] The nucleic-acid fragment according to claim 2 to 9 which has at least one side of the part in which an indicator and solid phase support, and association are possible.
[Claim 11] A nucleic-acid fragment given in either of claims 10 which have at least one side of the part in which an indicator and solid phase support, and association are possible in a five prime end.
[Claim 12] The nucleic-acid fragment according to claim 10 or 11 at least whose one side of the part in which an indicator and solid phase support, and association are possible is either biotin residue, a 2,4-dinitrophenyl radical and digoxigenin residue.

[Claim 13] Vibrio One share (FERM P-14643) of sp.KB, One share (FERM BP-5352) of JM, Pseudomonas cepacia KK01 share (FERM BP-4235), Pseudomonas One share (FERM P-14726) of sp.TL, Two shares (FERM P-14642) of Alcaligenessp.TL, and Pseudomonas alcaligenes As a candidate for detection chosen from two shares (FERM BP-5354) of KB(s) It is the nucleic-acid fragment which has a base sequence as a primer for taking out a specific base sequence using the elongation reaction on the basis of a primer in the gyrB gene of ** 1 strain. The base sequence for elongation which enables elongation from the partial array of a proper, or the edge field of the complementary array at the gyrB gene made applicable [that was chosen from all the base sequences of the gyrB gene made applicable to detection / this] to detection, Or the nucleic-acid fragment characterized by having the variation array acquired within limits which do not spoil the elongation function of this base sequence for elongation by carrying out insertion, the deletion, or the permutation of one or more bases to this base sequence for elongation.
[Claim 14] The strain as said candidate for detection is Vibrio. Nucleic-acid fragment according to claim 13 with which it is one share (FERM P-14643) of sp.KB, and the gyrB gene has the base sequence of array number:1.
[Claim 15] The nucleic-acid fragment according to claim 13 with which the strain as said candidate for detection is one share (FERM BP-5352) of JM, and the gyrB gene has the base sequence of array number:2.
[Claim 16] The strain as said candidate for detection is Pseudomonas. cepacia Nucleic-acid fragment according to claim 13 with which it is KK01 share (FERM BP-4,235) and the gyrB gene has the base sequence of array number:3.
[Claim 17] The strain as said candidate for detection is Pseudomonas. Nucleic-acid fragment according to claim 13 with which it is one share (FERM P-14726) of sp.TL, and the gyrB gene has the base sequence of array number:4.
[Claim 18] The strain as said candidate for detection is Alcaligenes. Nucleic-acid fragment according to claim 13 with which it is two shares (FERM P-14642) of sp.TL, and the gyrB gene has the base sequence of array number:5.
[Claim 19] The strain as said candidate for detection is Pseudomonas. alcaligenes Nucleic-acid fragment according to claim 13 with which it is two shares (FERM BP-5354) of KB(s), and the gyrB gene has the base sequence of array number:6.
[Claim 20] The nucleic-acid fragment according to claim 13 to 19 which is ten or more bases and has the die length to the same number of bases as said gyrB gene.
[Claim 21] The nucleic-acid fragment according to claim 13 to 20 which has at least one side of the part in which an indicator and solid phase support, and association are possible.
[Claim 22] The nucleic-acid fragment according to claim 21 which has at least one side of the part in which an indicator and solid phase support, and association are possible in a five prime end.
[Claim 23] The nucleic-acid fragment according to claim 21 or 22 at least whose one side of the part in which an indicator and solid phase support, and association are possible is either biotin residue, a 2,4-dinitrophenyl radical and digoxigenin residue.
[Claim 24] In the detection approach of a microorganism of detecting a specific base sequence using a hybridization method using a probe to this microorganism for detection contained in the nucleic acid which the microorganism for detection has Said microorganism for detection is Vibrio. One share (FERM P-14643) of sp.KB, One share (FERM BP-5352) of JM, Pseudomonas cepacia KK01 share (FERM BP-4235), Pseudomonas One share (FERM P-14726) of sp.TL, Two shares (FERM P-14642) of Alcaligenessp.TL, and Pseudomonasalcaligenes It is 1 strain chosen from two shares (FERM BP-5354) of KB(s). The detection approach of the microorganism characterized by including a base sequence specific to said this microorganism for detection in a gyrB gene.
[Claim 25] The detection approach of a microorganism according to claim 24 that said probe is a nucleic-acid fragment according to claim 2 to 12.
[Claim 26] In the detection approach of the microorganism by amplifying a specific base sequence by the elongation reaction using a primer to this microorganism for detection contained in the nucleic acid which the microorganism for detection has, and detecting the amplified base sequence Said microorganism for detection is Vibrio. One share (FERM P-14643) of sp.KB, One share (FERM BP-5352) of JM, Pseudomonas cepacia KK01 share (FERM BP-4235), Pseudomonas One share (FERM P-14726) of sp.TL, Two shares (FERM P-14642) of Alcaligenessp.TL, and

`Pseudomonasalcaligenes It is 1 strain chosen from two shares (FERM BP-5354) of KB(s). The detection approach of the microorganism characterized by including a base sequence specific to said this microorganism for detection in a gyrB gene..

[Claim 27] The detection approach of a microorganism according to claim 26 that said primer is a nucleic-acid fragment according to claim 8 to 13.

[Claim 28] The detection approach of a microorganism according to claim 26 or 27 using the PCR method using two primers which specify the field which said elongation reaction elongates.

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NOTICES *

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention] This invention relates to a new nucleic-acid fragment and its application. In detail, it is related with the partial array of a nucleic-acid fragment available as the primer or probe for detecting a specific microorganism, and its configuration base sequence, and the detection approach of a specific microorganism using these.
[0002]
[Description of the Prior Art] In recent years, the environmental pollution by chlorinated organic compounds, such as aliphatic hydrocarbon, such as an aromatic compound, paraffin, and a naphthene, or a trichloroethylene, etc. poses a problem, the already polluted environment is purified, and establishment of the technique restored in the original condition is called for strongly. Although the various physicochemical technique is performed as this environmental restoration technique, the method of performing mere extract of a pollutant and recovery is main, and it cannot necessarily be said from a viewpoint of cost, operability, the amount of dropping energy, the processing range, etc. that it is a practical technique in many cases.
[0003] Then, processing using a microorganism is expected to this physicochemical technique as what can offer the restoration approach of a practical contamination environment. Here, although many of pollutants are the synthetic material used by the chemical industry etc., and there are many things without the easy processing since it is seldom decomposed by the microorganism which inhabits a nature, many strain which can disassemble difficulty resolvability compounds which have caused soil pollution, such as aromatic hydrocarbon and a chlorinated organic compound, has been separated in recent years.
[0004] Especially development of a trichloroethylene decomposition bacillus is furthered briskly, spraying to the soil of the recombination strain which modified the gene so that it seemed that the decomposition activity is raised or the inductor for guiding the production and secretion of a trichloroethylene dialytic ferment in a decomposition bacillus becomes unnecessary, i.e., dialytic ferment activity might be acquired without an inductor etc. is considered, and it is coming for the first time.
[0005] For example, the bacteria belonging to a Pseudomonas group are gram-negative obligatory aerobic nature Bacilli, it has the capacity which metabolizes a very variegated organic compound, excelling especially in the resolution of various aromatic compounds is known, and many strain which can disassemble difficulty resolvability compounds which have caused soil pollution, such as aromatic hydrocarbon and a chlorinated organic compound, has been separated.
[0006] Here, this invention persons are Vibrio. One share (FERM BP-14643) of sp.KB, One share (FERM BP-5352) of JM, Pseudomonascepacia KK01 share (FERM BP-4235), Pseudomonas One share (FERM P-14726) of sp.TL, Alcaligenes Two shares (FERM P-14642) of sp.TL, and Pseudomonas alcaligenes Two shares (FERM BP-5354) of KB(s) are showing clearly to be able to decompose a trichloroethylene. The environmental purification technique in which such a microorganism was used is spread, and it is in a pan, In order to fix this technique as an effective technique practical and socially, it has been an important technical problem to fully grasp the dominance of the bacillus in the activity of the bacillus in the soil which introduced them, growth, propagation, and survival, i.e., soil etc., a growth situation, etc. with development of the bacillus

excellent in decomposition capacity etc. In order to solve these technical problems, the establishment of technique which can detect the target bacillus alternatively is improper decision.
[0007] The various isolation culture using the culture medium which can isolate a specific microorganism as the detection approach of various microorganisms is used. Alternative culture by the special culture medium alternatively applicable to the microorganism which it is going to separate generally is performed in many cases. Although it is widely used since this approach is simple, only the microorganism made into the purpose may necessarily be cultivated alternatively. For example, in the case of the possible above-mentioned trichloroethylene decomposition strain, disassembly of a phenol also makes a simple index coloring of the yellow of the 2-hydroxy muconic acid semialdehyde which is a degradation product by the catechol -2 of a catechol, and 3-oxygenase, and the check of the existence is possible for it. However, other microorganisms in which a metabolic turnover is possible may exist a catechol according to meta-cleavage, and it cannot make only yellow coloring into the index strictly, but it is necessary to inspect detailed morphological or biochemical description. Here, much skill and experience were required for detecting the microorganism made into the purpose from morphological or biochemical description, and, moreover, the technique had a problem in respect of [, such as it being complicated and taking time amount,] various practical use.
[0008] Moreover, although the approach of carrying out the label of the specific antibody to the microorganism which should be detected by radioisotope or the fluorochrome, and using for detection of this microorganism was learned, this approach was what has a difficulty in the point which requires time and effort for obtaining an antibody very much, and the point that the singularity of an antibody varies greatly for every time of that preparation.
[0009] The approach of detecting by using for a living thing kind the oligonucleotide primer or probe which has a complementary array for the array of a specific nucleic acid at it as what is replaced with these approaches is announced. Especially, RIBOZOMARU RNA (rRNA) is an indispensable cell constituent in a living thing, and the structure is saved comparatively well in the process of evolution of a living thing. About 16SrRNA(s), the research is progressing also in rRNA, and the base sequence has been identified about many living thing kinds. It is known by 16SrRNA(s) for various living things that there are a field saved in common and a variable region where an array changes with living thing kinds, and detection of the microorganism which used the DNA primer or probe corresponding to this variable region, and a method of identification have been developed in recent years.
[0010]
[Problem(s) to be Solved by the Invention] In order to make processing conditions and a condition for growth the optimal when performing purification processing of the pollutant of the soil middle class using a specific microorganism as mentioned above, it is necessary to get to know the dominance and growth situation in the soil middle class of the microorganism. For that purpose, although the approach of detecting a microorganism specifically and measuring it is required Detection of various old microorganisms is culture by the selective medium which makes the specific matter a single carbon source, and the thing based on antibody technique etc. It cannot necessarily be satisfied in respect of the time amount which the singularity, sensibility, simplicity, and detection take, and the more practical approach for carrying out monitoring of the behavior of the microorganism in the soil middle class on real time in many cases was searched for.
[0011] Since this invention corresponds to such a demand, it accomplishes, and the purpose is in offering detection and the approach of carrying out counting about quickness and a specific microorganism with sensibility simple and sufficient specific moreover.
[0012] Other purposes of this invention improve further the detection technique using rRNA currently conventionally used widely, and are to offer the technique which makes possible detection of a still more specific and alternative microorganism, and counting.
[0013] That is, since the detection using 16rRNA has the field which is saved in common and is by various living things, and the variable region where an array changes with living thing kinds, it sets up the part made into this variable region by recognition by the primer or the probe, and is based on the principle of detecting the target microorganism. Here, about rRNA, molecular evolution is comparatively loose and it is mentioned as a technical problem between distant relative groups that a

certain rise of the background [although it is and high selectivity and singularity are acquired between sowings, selectivity and singularity fall between the kinds of a close relationship or in the interval between roots which can be set of the same kind, and] at the time of detection, the fall of the dependability of the detection data based on it, etc. arise. It is **-like that the detection approach of having higher selectivity and singularity is especially desired strongly in an environment like soil where a miscellaneous microorganism exists variously.
[0014]
[Means for Solving the Problem] this invention persons chose the gyrB gene as a gene which has the field saved common to various living things, and the variable region where an array changes with living things. As compared with rRNA, a gyrB gene is known for the molecular evolution rate of a variable region being large, and is. That is, as compared with the case where the recognition field of a primer or a probe is set up all over the variable region of rRNA, it becomes possible by setting up all over this variable region in the recognition site in a primer or a probe to raise selectivity and singularity notably.
[0015] The base sequence which the gyrB gene concerning this invention has The array number of an array table : by it being shown in 1-6 and this base sequence having been solved Vibrio One share (FERM P-14643) of sp.KB, One share (FERM BP-5352) of JM, Pseudomonas cepacia KK01 share (FERM BP-4235), Pseudomonas One share (FERM P-14726) of sp.TL, Alcaligenes Two shares (FERM P-14642) of sp.TL, and Pseudomonas alcaligenes It becomes possible to acquire the useful information for an improvement of the detection approach of two shares (FERM BP-5354) of KB(s).

[0016] In addition, such strain is the above-mentioned trust numbers, international deposition (thing of a FERM BP-number) of [under domestic deposition (thing of a FERM P-number) or Budapest Treaty] is carried out to National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, the Ministry of International Trade and Industry, and the deposition day is as follows.
One share () of KB [FERM] P-14643: One share () of November 15, Heisei 6 JM [FERM] BP-5352:January19, Heisei 7 KK01 share (FERM BP-4235): -- March 11, Heisei 4 TL1 share (FERM P-14726): -- January 10, Heisei 7 TL2 share (FERM P-14642): -- Heisei 6 -- year 11 month 15 day KB2 share () [FERM] BP-5354 : the nucleic-acid fragment concerning this invention useful as a probe useful to detection by the hybridization method of such strain on November 15, Heisei 6 a) All the base sequences or the complementary array of those of the gyrB gene made applicable to detection, As opposed to all these base sequences a or the partial array b b) -- the gyrB gene made applicable [that was chosen from these all base sequences / this] to detection -- the partial array of a proper, or the complementary array and c -- It can prepare as a nucleic-acid fragment which has the variation array acquired by performing insertion, the deletion, or the permutation of one or more bases within limits which do not spoil the function as a probe for gyrB gene detection, or its complementary array.
[0017] Moreover, the nucleic-acid fragment concerning this invention useful as a primer useful to the detection using the PCR method of such strain The base sequence for elongation which enables elongation from the partial array of a proper, or the edge field of the complementary array at the gyrB gene made applicable [that was chosen from all the base sequences of the gyrB gene made applicable to detection / this] to detection, Or it can prepare as a nucleic-acid fragment which has the variation array acquired within limits which do not spoil the elongation function of this base sequence for elongation by carrying out insertion, the deletion, or the permutation of one or more bases to this base sequence for elongation. When the elongation reaction is used, the magnification reaction by the PCR method which specifies an elongation field by two sorts of primers preferably is used preferably.
[0018] Furthermore, in the detection approach of a microorganism of detecting a specific base sequence using a hybridization method using a probe to this microorganism for detection contained in the nucleic acid which the microorganism for detection has, the detection approach of the microorganism in this invention is the strain which said microorganism for detection mentioned above, and is characterized by to include a base sequence specific to said this microorganism for detection in a gyrB gene.

[0019] Moreover, in this invention, in the detection approach of a microorganism of detecting a specific base sequence using a hybridization method using a probe to this microorganism for detection contained in the nucleic acid which the microorganism for detection has, the detection approach of other microorganisms is each strain which said microorganism for detection mentioned above, and is characterized by to include a base sequence specific to said this microorganism for detection in a gyrB gene.
[0020] According to this invention, it becomes a microorganism for detection detectable [the microorganism for detection in good singularity and selectivity] as a specific (characteristic) base sequence by having chosen the variable region or its part also in the gyrB gene.
[0021]
[Embodiment of the Invention] Hereafter, this invention is further explained to a detail.
(Decision of the variable region base sequence of a gyr gene) The microorganism for detection in this invention is each strain mentioned previously, and the base sequence of these gyrB genes compares the gyrB base sequence of known Gram negative bacterium, and can determine it according to a conventional method using two sorts of primers chosen from the consensus sequence field. In the decision of this base sequence, it is ABIPRISM to for example, Parkin Elman's DNA sequencer list. Dye Primer Cycle Sequencing Kit can be used.
[0022] Array number: (Nucleic-acid fragment) The nucleic-acid fragment which has the complementary array of the base sequences shown by 1-6 and these base sequences can be used as a probe in detection of the gyrB gene which uses a hybridization method. Furthermore, an array number: The part which fills the function as a probe which enables alternative detection of a gyrB gene can be chosen from the complementary array of the base sequences shown by 1-6, and these base sequences, and the nucleic-acid fragment which has the base sequence of this part can also be used as a probe in detection of the gyrB gene using a hybridization method. Moreover, the base sequence which the nucleic-acid fragment for these probes has may have the variation by insertion, the deletion, or the permutation of one or more bases within limits which do not spoil the function made into the purpose as a probe.
[0023] On the other hand, when performing detection of a gyrB gene using the elongation reaction of the nucleic-acid chain from a primer, the primer which can amplify a partial base sequence characteristic of the whole gyrB gene or a gyrB gene can be chosen and used. As this primer, the nucleic-acid fragment which has the partial array which has a function as a primer which enables alternative detection of a gyrB gene can be chosen and used from the complementary array of the base sequences shown by array number:1-6, and these base sequences. It is set up according to the magnification conditions in PCR etc. in that case whether the nucleic-acid fragment which has the array of the edge of the part which wants to amplify the base sequence which a gyrB gene has as it is is used, or the nucleic-acid fragment which has the complementary array is used. Furthermore, the base sequence of the primer chosen in this way may have the variation by insertion, the deletion, or the permutation of one or more bases within limits which do not spoil the function made into the purpose as a primer. In addition, in using variation, even if it makes it near the three-dash terminal considered to have big effect on the elongation reaction of the nucleic-acid chain from a primer not have variation or there is, stopping to the minimum is desirable, and it makes it have variation near a five prime end more suitably.
[0024] In selection of the partial base sequence made to hold in the nucleic-acid fragment used as such a probe or a primer, it is specific in the gyrB gene of the microorganism for detection, and it is desirable to choose a part with little homology as the bacteria of Pseudomonas groups, such as other bacteria, for example, P.putida, and P.aeruginosa, the Alcaligenes group bacteria well separated in soil, Xanthomonas group bacteria, Agrobacterium group bacteria, Enterobacteraceas group bacteria, the Acinetobacter bacteria, etc. When the die length of these nucleic-acid fragments can use the thing of the die length from ten bases to the gyrB gene overall length for detection when using as a probe, and using it as a primer, it can use the thing of the die length of the number of 10 - 50 bases. What was mentioned as the example mentioned later as a desirable nucleic-acid fragment can be mentioned.
[0025] These nucleic-acid fragments may carry out chemosynthesis of all arrays or the part of those to the example which can prepare by the approach of appropriate arbitration, for example, is

mentioned later according to the approach of a publication. Or it is also possible to amplify by the PCR method using the primer which carried out chemosynthesis, and to use a magnification fragment as a probe as it is. Furthermore, the direct nucleic-acid fragment considered as a request using a suitable restriction enzyme from the microorganism as a candidate for detection can be cut down, or a cDNA fragment can be prepared from a corresponding RNA fragment, and this can also be used. Or into suitable hosts, such as E.coli, vectors, such as a plasmid, can be used, cloning of the part which has the target nucleic-acid fragment can be carried out, and it can be made to be able to amplify by growth of a bacillus, and after growth, vectors are collected, and from now on, it can start and can also obtain.

[0026] As for the nucleic-acid fragment available as such a primer or a probe, the part in which an indicator or solid phase support, and association are possible may be introduced if needed. When performing detection using a primer here, if the location which can introduce the part in which an indicator or solid phase support, and association are possible is a location which does not bar the elongation reaction of a primer, it is good anywhere, but if possible, a five prime end is desirable. Moreover, although the location which can introduce the part in which an indicator or solid phase support, and association are possible can mention the hydroxyl-group parts of a three-dash terminal or a five prime end, a base part, a phosphoric-acid diester part, etc. when performing detection using a probe, it is desirable to make it not become the hindrance of hybridization in consideration of a base sequence, die length, etc. of a probe.

[0027] (Part in which an indicator and solid phase support, and association are possible) When using the above-mentioned nucleic-acid fragment as a probe or a primer, as an indicator for detection, whichever of the radioactive substance and an inactive substance may be used. As a nonradioactive indicator, fluorescent materials, such as a fluorescein derivative, a rhodamine, and its derivative, the chemistry generating matter, the delayed fluorescence matter, etc. are mentioned as a thing in which a direct indicator is possible. Moreover, it is also possible to detect an indicator indirectly using the matter specifically combined with an indicator. A biotin, hapten, etc. are mentioned as such an indicator, and when it is a biotin, the antibody to which a horse mackerel pin or streptoavidin is specifically combined with this in the case of hapten can be used. As hapten, a compound, digoxigenin, etc. which have a 2,4-dinitrophenyl radical, for example can be used. Each can introduce these indicators into a probe or a primer combining two or more sorts independent or if needed.

[0028] When the specific fragment of nucleic acids, such as sandwiches hybridization, needs to be specifically combined with solid phase support, as long as association with this support and a selection target is possible for the part in which solid phase support and association are possible, there may be why. For example, haptens which have a biotin or a fluorescein, and a 2,4-dinitrophenyl radical, such as a compound and digoxigenin, are mentioned, and each can introduce these into a probe or a primer combining two or more sorts independent or if needed.

[0029] (The detecting method using a probe) A specific microorganism is detectable using at least one sort of a nucleic-acid fragment which has a function as a probe mentioned above. As a hybridization method which can be used for the detection using a probe, they are dot hybridization, Southern hybridization, and in. situ Hybridization etc. can use the nucleic-acid fragment which exists and has the indicator mentioned above, and can perform hybridization as a conventional method. It is in similarly. situ Also in hybridization, it is possible to present detection with the above-mentioned nucleic-acid fragment. Moreover, the approach based on sandwiches hybridization is developed for simplification of hybridization actuation, and detection of the microorganism for detection is possible also in these approaches using the above-mentioned nucleic-acid fragment.

[0030] (The detecting method using a primer) A specific microorganism is detectable using at least one sort of a nucleic-acid fragment which has a function as a primer mentioned above. PCR (Polymerase Chain Reaction) which amplifies the minute amount nucleic-acid fragment in a sample as a more desirable example by the gene amplification reaction by two sorts which are mentioned later of different primers -- it is the detecting method using law. Here as a fundamental gestalt of two sorts of primers For example, that to which no qualification is carried out for two sorts of primers, the thing into which the part in which an indicator or solid phase support detectable to at least one side, and association are possible was introduced among two sorts of primers, That by which the

indicator was introduced into one side among two sorts of primers, and the part in which solid phase support and association are possible was introduced into another side, the thing into which the part which two sorts of primers combine [solid phase support and] was introduced are mentioned.
[0031] The following processes can perform the primer of the microorganism by this invention, and the detection approach using two sorts of primers preferably.
(1) Prepare the sample which detected the existence of the existence of the microorganism as a candidate for detection, perform crushing processing of the fungus body in a sample if needed [(2)], add a primer into (3) samples, and start from the primer combined with the nucleic acid in a sample. The elongation reaction of a nucleic-acid chain is performed by using the base sequence by the side of the nucleic acid in a sample as mold, and detection actuation about the elongation reactant obtained at (4) processes (3) is performed.
[0032] It is also possible to use the approach of using the approach usually used [hybridization / electrophoresis or] for detection of a magnification **** nucleic-acid fragment by the primer elongation reaction here, introducing the separate indicator into each primer in the magnification reaction of a gene, making a product sticking to solid phase support after magnification reaction termination, and detecting this product alternatively etc. here -- as solid phase support -- a polystyrene ball, an agarose bead, the poly acrylic bead, a latex, and a micro titer -- what introduced streptoavidin for which the prehension introduced into the primer is possible, an antibody, etc. into solid phase base materials, such as a well, is mentioned. For example, what is necessary is just to use the support which combined the antibody to each with solid phase for catching the elongation reaction from the primer into which the fluorescein etc. was introduced using the support which combined streptoavidin with solid phase for catching the PCR product from a primer with which the biotin was introduced. Furthermore, the target nucleic acid can also be judged simple by the existence of condensation or precipitation by making solid phase support into a particle.
[0033] Moreover, after contacting the reactant which performed the elongation reaction to solid phase support using what introduced the indicator into the primer of another side for the part in which solid phase support and association with one primer are possible, the approach of carrying out washing removal of the impurity with a suitable solvent is devised, in a form with a target, it is fixed to solid phase support and the purpose nucleic acid is detected specifically. Actual detection of these indicators should just use general technique according to the target which uses it. For example, what is necessary is color for what is necessary to be just to measure activity as it is, if an indicator is radioisotope, and to make it react with a substrate with AMPPD etc. using antibody-enzyme combination etc., and for a fluorescence-means just to detect, if it is a biotin, for example and is avidin-enzyme combination and hapten.
[0034] Moreover, although it has in magnification of a gene and various approaches are devised about ******, reflective conditions, etc., the nucleic-acid fragment in this invention has sufficient die length and a sufficient base sequence to use for the various PCR methods, and can detect a specific microorganism by using this.
[0035]
[Example] Example 1 (decision of the variable region base sequence of a gyrB gene)
The base sequence of the gyrB gene of known various gram negatives was compared, and the primer shown below from a consensus sequence field was set up.
Primer A: 5'CAGGAAACAGCTATGACCAARMGICCNGGIATGTAYATHGG3' (array number: 7)
(here -- tag array:M13r for base sequence determination in CAGGAAACAGCTATGACC -- it is compatible.)
Primer B: 5'TGTAAAACGACGGCCAGTAAYTTNGGNTCNGGNACYTT3' (array number: 8)
(here -- tag array:M13-21 for base sequence determination in TGTAAAACGACGGCCAGT -- it is compatible.)
Vibrio One share (FERM P-14643) of sp.KB, one share (FERM BP-5352) of JM, Pseudomonas cepacia KK01 share (FERM BP-4235), Pseudomonas One share (FERM P-14726) of sp.TL, Alcaligenes Two shares (FERM P-14642) of sp.TL, and Pseudomonas alcaligenes About each of the nucleic acid obtained from two shares (FERM BP-5354) of KB(s) Magnification by PCR is performed using the two above-mentioned sorts of primers with a conventional method. It is the

DNA sequencer and ABIPRISM of PerkinElmer, Inc. about the base sequence in the amplified nucleic-acid fragment. Dye Primer Cycle It determined using SequencingKit. The terms and conditions and actuation in base sequence determination were performed according to the manual.

[0036] In this way, the determined base sequence is a base sequence (array number: 1-6) of the gyrB gene of each stock. In addition, analysis of a base sequence performed the check of being a gyrB gene.

[0037] Example 2 (KB preparation of the primer for one shares)

The primer into which the part in which an indicator or solid phase support, and association are possible is introduced by the chemosynthesis method shown below if needed was prepared. first, the thing into which the part in which association with an indicator and solid phase support is possible puts, and a gap is not introduced, either -- a DNA automatic composition machine model (PerkinElmer) -- using -- phospho friend DAIDO -- it compounded on 0.2micromol scale by law, and the OPC cartridge (PerkinElmer) refined.

[0038] Moreover, an indicator or solid phase support, and the primer into which the combinable part is introduced were compounded as an oligonucleotide which introduced the amino group into the five prime end first, and introduced after that the part in which an indicator or solid phase support, and association are possible using the suitable reagent. The example is shown below.

[0039] Oligonucleotide:5'CGCCAAGGGCGACGTACAGAACC3' which introduced the amino group into the five prime end (array number: 9)

After ****** added the last base (in this case, G) to the five prime end by the synthetic reaction which was mentioned above, it was performed by adding the amino link II (PerkinElmer) further, and the OPC cartridge refined it after synthetic termination. Moreover, biotin-ization was performed by [as being the following]. In 10micro of amination oligonucleotide water solutions l of 1O.D., it is 1M. The DMF solution of the BIOCHINIRU-N-hydroxy Succin imide ester (BRL) of NaHCO3 water-solution 10microl, water 30microl, and 20micro g/mu l was 50microl Added, and it was left at the room temperature after mixing. It applies to the gel filtration which made sephadex G-50 support 4 hours after, and is 50mM. It was eluted with the TEAB (heavy carbonic acid triethyl ammonium) buffer solution (pH7.5), the first peaks were collected, and it dissolved in TE buffer solution (pH8.0) after hardening by drying.

[0040] Oligonucleotide:5'GTGCAGCACGCTCTTGGAGCG3' which introduced the dinitro radical (DPN) into the five prime end (array number: 10)

Composition and purification were performed like the time of a ** biotin indicator as an oligonucleotide which introduced the amino group into the five prime end first. Thus, in 180micro of amination oligonucleotide water solutions l of obtained 2O.D., it is 1M. 20micro of NaHCO3 water solutions l was added, and 100micro of ethanol solutions l of dinitro Fluon benzene was added to this 5% (v/v), and at 37 degrees C, it warmed for 2 hours and reacted. Gel filtration performed purification like the biotin ** oligonucleotide, and it was dissolved in TE buffer solution (pH8.0) after hardening by drying.

[0041] Example 3 (KB preparation of the probe for one shares)

Oligonucleotide:5'GCGCCACCGACAAGCGCGGCACTGAAGTCCA3' which introduced the biotin indicator into the three-dash terminal (array number: 11)

It prepared. namely, the 3'Biotin-ONCPG column (CLONTECH) of 0.5micromol scale with which the biotin indicator of the three-dash terminal is carried out beforehand -- using -- phospho friend DAIDO -- the oligonucleotide was compounded by law and it dissolved in TE buffer solution (pH8.0) after purification and hardening by drying using the OPC cartridge with the conventional method.

[0042] Example 4 (evaluation of the singularity of KB1 share primer)

One share of KB, one share of JM, P.cepacia One share of KKO, a P.putidaBH stock, P.aeruginosa IFO 3080 shares, P.fluorescens IFO 14160 shares, Alcaligenes faecalisIFO 14479 shares, Xanthomonas 14161 shares of maltophiliaIFO(s), Enterbacter cloacae 13535 shares of IFO(s), E.coli Ten sorts of bacteria of 109 shares of JM are used, and it is a conventional method. From each bacteria, what prepared DNA was made into the sample and the PCR method estimated the singularity of a primer.

[0043] 2 sorts of primers: which prepared the primer in the example 2 --

5'CGCCAAGGGCGACGTACAGAACC 3'5 -- 'GTGCAGCACGCTCTTGGAGCG3' was used. PCR was performed in the following reaction solution presentation lists on conditions.

[0044] 5microl and Sample DNA were added [the above-mentioned primer of 50 pmol/mu l concentration] for the dNTP mixed solution of attachment of the reaction buffer solution of attachment in 1micro every [l] and an enzyme in 5microl and an enzyme 10 ngs, respectively, water was added further, and the reaction solution whole quantity was set to 50microl. After having added AmpliTaqDNApolymerase (TAKARA SHUZO) to this 1 unit, warming at 90 degrees C and holding for 5 minutes, for 90 degree C and 60 seconds, for 55 degree C and 45 seconds, 30 cycles which made 1 cycle 72 degree C and 90 seconds were reacted, and it was kept warm for 5 minutes at 72 more degrees C after the reaction. 10microl was isolated preparatively from 50microl after the reaction, agarose electrophoresis and ethidium-bromide dyeing were performed, and the magnification nucleic-acid chain was detected.

[0045] Consequently, only in one share of KB, one clear band has been checked to the die length of about 390 base pairs expected. Here, in nine sorts of other strain, any magnification nucleic-acid chains were undetectable at all.

[0046] Example 5 (detection using a KB1 share primer (1))

DNA was prepared from ten kinds of each bacteria like the example 4. PCR was presented with this by the following reaction solution presentations and the reaction condition.

[0047] primer:5'-Biotin-CGCCAAGGGCGACGTACAGAACC 3'5 of the 20 pmol/mu l concentration prepared in the example 2, respectively one share of KB added the dNTP mixed solution of attachment of every [1microl] and the reaction buffer solution of attachment in an enzyme in 5microl and an enzyme for '-DNP-GTGCAGCACGCTCTTGGAGCG3', and added 10pg (s) for 2microl and Sample DNA 10 ngs about other bacteria, water was added further, and the reaction solution whole quantity was set to 50microl. After having added AmpliTaqDNApolymerase (TAKARA SHUZO) to this 1 unit, warming at 90 degrees C and holding for 5 minutes, for 90 degree C and 60 seconds, for 55 degree C and 45 seconds, 35 cycles which made 1 cycle 72 degree C and 90 seconds were reacted, and it was kept warm for 5 minutes at 72 more degrees C after the reaction. This reaction mixed liquor was applied to the spin column, and the unreacted primer was removed. To a streptoavidin fixed microplate, it is 0.15M. NaCl and the Tris-Cl buffer solution (pH7.5) which contains Tween20 0.05% are 100microl Added, the above-mentioned mixed liquor after reacting to this was 10microl Added, and the 500micro of the above-mentioned buffer solutions l washed 3 times after neglect for 30 minutes at the room temperature. What diluted the alkaline phosphatase indicator anti-DNP antibody with the above-mentioned buffer solution 2000 times was 100microl Added to this, and the 500micro of the above-mentioned buffer solutions l washed 3 times after neglect for 30 minutes at the room temperature. p-nitrophenyl phosphoric-acid solution which dissolved in 1M diethanolamine buffer solution by the concentration of 4mg/ml was 100microl added to this, and the absorbance of 405nm was measured after neglect using the microplate reader for 30 minutes at the room temperature. Consequently, only in one share of KB, significant absorption was able to be checked as compared with the background. Here, in nine sorts of other strain, it stopped at absorption of background extent being shown.

[0048] Example 6 (detection using a KB1 share primer (2))

DNA was prepared from one share of KB like the example 4. PCR was presented with this by the following reaction solution presentations and the reaction condition. namely, primer:5'-Biotin-CGCCAAGGGCGACGTACAGAACC 3'5 of the 20 pmol/mu l concentration prepared in the example 2 -- '-DNP-GTGCAGCACGCTCTTGGAGCG3' -- respectively -- the dNTP mixed solution of attachment of every [1microl] and the reaction buffer solution of attachment in an enzyme in 5microl and an enzyme -- as 2microl and a sample DNA prepared from one share of KB was added 10 fg 100 fg 1 pg 10 pg, water was added further, and the reaction solution whole quantity was set to 50microl. After having added AmpliTaqDNApolymerase (TAKARA SHUZO) to this 1 unit, warming at 90 degrees C and holding for 5 minutes, for 90 degree C and 60 seconds, for 55 degree C and 45 seconds, 40 cycles which made 1 cycle 72 degree C and 90 seconds were reacted, and it was kept warm for 5 minutes at 72 more degrees C after the reaction. This reaction mixed liquor was applied to the spin column, and the unreacted primer was removed.

[0049] To a streptoavidin fixed microplate, it is 0.15M. NaCl and the Tris-Cl buffer solution (pH7.5)

which contains Tween20 0.05% are 100microl Added, the above-mentioned mixed liquor after reacting to this was 10microl Added, and the 500micro of the above-mentioned buffer solutions 1 washed 3 times after neglect for 30 minutes at the room temperature. What diluted the alkaline phosphatase indicator anti-DNP antibody with the above-mentioned buffer solution 2000 times was 100microl Added to this, and the 500micro of the above-mentioned buffer solutions 1 washed 3 times after neglect for 30 minutes at the room temperature. p-nitrophenyl phosphoric-acid solution which dissolved in 1M diethanolamine buffer solution by the concentration of 4mg/ml was 100microl added to this, and the absorbance of 405nm was measured after neglect using the microplate reader for 30 minutes at the room temperature. Consequently, the amount of DNA was able to check significant absorption about the thing of 10pg(s), 1pg, and 100fg as compared with the background. Here, the amount of DNA stopped at absorption of background extent being shown about the thing of 10fg(s).

[0050] Example 7 (detection using a KB1 share probe (1))

DNA was prepared from ten kinds of various bacteria like the example 4. The blot of each DNA was carried out to the nylon film (Tropilon-45, product made from Tropix) every [1micro / 1] after alkali denaturation using dot blot equipment (BRL). The nylon film was put into the vinyl back after 2-hour desiccation at 80 degrees C, 3ml (6xSSC, 5x Denhardt's solution, 0.5%SDS, 100microg [/ml] denaturation salmon sperm DNA) of pre hybridization solutions was added, and pre hybridization was performed at 60 degrees C for 1 hour. One hybridization 3m solution (the biotin indicator RIGONUKUREOCHIDOPU lobe prepared in the example 3 in the pre hybridization solution: what added 5'GCGCCACCGACAAGCGCGGCACTGAAGTCCA-Biotin3' 100 ng after thermal denaturation) was added to this, and hybridization was performed to it at 60 degrees C for 2 hours. the nylon film was taken out from the vinyl back and it washed every 3 times during 5 minutes at 60 degrees C using 6xSSC and 0.5%SDS solution. Detection went according to the attached protocol using the chemiluminescence method by alkaline phosphatase indicator streptoavidin and AMPPD using the Southern light (Tropix).

[0051] Consequently, the very strong electropositive reaction has been checked only about what carried out the blot of the 1 share of KBDNA. Here, in nine sorts of other strain, the electropositive reaction was undetectable.

[0052] Example 8 (detection using a KB1 share probe (2))

Ten sorts of various bacteria of a publication were cultivated with the conventional method in the example 4. 0.1M Fungus body suspension was prepared so that each fungus body might become [ml] in 2x107 pieces /after washing a fungus body using said buffer solution with the phosphoric-acid NATORIMU buffer solution (pH8.0). 6% of formaldehyde solution was 50microl Added to 50microl of this fungus body suspension, and the fungus body was fixed, and 30microl was dropped at 0.1% gelatin and the slide glass which carried out the coat by the chrome alum 0.001%, and it was air-dry. Pure water washed, after soaking the slide glass which fixed the sample for 10 minutes in the methanol and 3% formaldehyde solution 90% and re-fixing a fungus body.

[0053] About the slide glass which performed the above-mentioned processing, it is 50mM. After dipping in 10 mMTris-Cl buffer solution (pH8.0) containing NaBH4 in the state of protection from light for 30 minutes at a room temperature, it washed and was air-dry with pure water. The biotin indicator oligonucleotide probe which prepared the probe in the example 3: What combined beforehand the streptoavidin by which the FITC (Fluorescein isothiocyanate) indicator was carried out to 5'GCGCCACCGACAAGCGCGGCACTGAAGTCCA-Biotin3' was used. This indicator probe was made into 5 ng(s)/ml concentration with the hybridization solution (the 0.1M Tris-Cl buffer solution (pH8.0), 0.75M NaCl, 5mM EDTA, 10% dextran sulfate, 0.2%BSA (Bovine Serum Albumin), 0.01% polyadenylic acid), and 30microl was dropped. The slide glass was put into the airtight container and 45 degrees C and the reaction of 1 hour were performed in the state of protection from light.

[0054] After a reaction. After washing the slide glass and being air-dry in the state of protection from light with the SET buffer solution (Tris-Cl, pH8.0, 0.2mM EDTA, 30mM NaCl), the Olympus incident light mold fluorescence microscope performed the speculum, and the existence of fluorescence was investigated. The excitation light source used the mercury lamp and observed by B excitation. Although fluorescence was accepted in the fungus body about one share of KB as a result

of the speculum, with other bacteria, fluorescence was unobservable.
[0055] Example 9 (preparation of the primer for JM1 shares)
Array number: Instead of 9 and 10, the oligonucleotide was compounded like the example 2 except using the following arrays.
5'GAATGCCTTGTCACGTAAGTTGG3' (array number: 12)
5'CACTGAAACAATCGCAGTTAAAC3' (array number: 13)
Example 10 (preparation of the probe for JM1 shares)
Array number: The oligonucleotide was compounded like the example 3 except using the following arrays instead of 11.
5'CGTATCGGTTGTGAATGCCTTGTCACGTAAGTTGGA3' (array number: 14)
Example 11 (evaluation of the singularity of the primer for one share of JM)
As sample strain One share of JM, P.cepacia One share of KKO, P.putida BH stock, P. aeruginosa IFO 3080 shares, P.fluorescens IFO 14160 shares, Alcaligenes faecalis IFO 14479 shares, Xanthomonas maltophilia 14161 shares of IFO(s), Enterbacter cloacae 13535 shares of IFO(s), E.coli Nine sorts of bacteria of 109 shares of JM are used. As a primer 5'-Biotin-GAATGCCTTGTCACGTAAGTTGG 3'5 -- magnification and its detection of the nucleic acid by PCR were performed like the example 4 except having used '-DNP-CACTGAAACAATCGCAGTT AAAC3'. Consequently, only in one share of JM, one clear band has been checked to the die length of about 600 base pairs expected. Here, in eight sorts of other strain, any magnification nucleic acids were not detected at all.
[0056] Example 12 (detection using the primer of one share of JM (1))
DNA was prepared from nine sorts of each strain like the example 11. Magnification of the nucleic acid by PCR and the amplified nucleic-acid fragment were detected like the example 5 except using the primer used in the example 11 about each DNA.
[0057] Consequently, only in one share of JM, significant absorption was able to be checked as compared with the background. Here, eight sorts of other strain stopped at absorption of background extent being shown.
[0058] Example 13 (detection using the primer of one share of JM (2))
DNA was prepared from one share of JM like the example 11. Magnification of the nucleic acid by PCR and the amplified nucleic-acid fragment were detected like the example 6 except using the primer used in the example 11 about each DNA.
[0059] Consequently, the amount of DNA was able to check significant absorption about the thing of 10pg(s), 1pg, and 100fg as compared with the background. Here, the amount of DNA stopped at absorption of background extent being shown about the thing of 10fg(s).
[0060] Example 14 (detection using the probe of one share of JM (1))
DNA was prepared from nine sorts of each strain like the example 11. Detection which used the hybridization method like the example 7 was performed except using probe:5'CGTATCGGTTGTGAATGCCTTGTCACGTAAGTTGGA-Biotin3' of the following prepared in the example 10 about each DNA. Consequently, the very strong electropositive reaction has been checked only about what carried out the blot of the 1 share of JMDNA. Here, in eight sorts of other strain, the electropositive reaction was undetectable.
[0061] Example 15 (detection using the probe of one share of JM (2))
DNA was prepared from nine sorts of each strain like the example 11. Detection which used the hybridization method like the example 8 was performed except using probe:5'CGTATCGGTTGTGAATGCCTTGTCACGTAAGTTGGA-Biotin3' of the following prepared in the example 10 about each DNA. Consequently, although fluorescence was accepted in the fungus body about one share of JM, in other strain, fluorescence was unobservable.
[0062] Example 16 (preparation of the primer for KK01 shares)
Array number: Instead of 9 and 10, the oligonucleotide was compounded like the example 2 except using the following arrays.
5'CGTGCTCGAGCAGGTGGACGGTGTG3' (array number: 15)
5'TTATCCTTCTCGCCGGTGGCGAAGAAAATC3' (array number: 16)
Example 17 (preparation of the probe for KK01 shares)
Array number: The oligonucleotide was compounded like the example 3 except using the following

arrays instead of 11.
5'GGACGGTGTGGAAGTGTCGCCGATGCTGGTGACCGGTGATA3' (array number: 17)
Example 18 (evaluation of the singularity of the primer for KK 01 shares)
As sample strain P.cepacia One share of KKO, P.putida BH stock, P. aeruginosa IFO 3080 shares, P.fluorescens IFO 14160 shares, Alcaligenes faecalis IFO 14479 shares, Xanthomonas maltophilia 14161 shares of IFO(s), Enterbacter cloacae 13535 shares of IFO(s), E.coli Nine sorts of bacteria of 109 shares of JM and one share of JM are used. As a primer 5'-Biotin-CGTGCTCGAGCAGGTGGACGGTGTG 3'5 -- magnification and its detection of the nucleic acid by PCR were performed like the example 4 except having used '-DNP-TTATCCTTCTCGCCGGTGG CGAAGAAAATC3'. Consequently, only in KK01 share, one clear band has been checked to the die length of about 320 base pairs expected. Here, in eight sorts of other strain, any magnification nucleic acids were not detected at all.
[0063] Example 19 (detection using a KK01 share primer (1))
DNA was prepared from nine sorts of each strain like the example 18. Magnification of the nucleic acid by PCR and the amplified nucleic-acid fragment were detected like the example 5 except using the primer used in the example 18 about each DNA.
[0064] Consequently, only in KK01 share, significant absorption was able to be checked as compared with the background. Here, eight sorts of other strain stopped at absorption of background extent being shown.
[0065] Example 20 (detection using a KK01 share primer (2))
DNA was prepared from KK01 share like the example 18. Magnification of the nucleic acid by PCR and the amplified nucleic-acid fragment were detected like the example 6 except using the primer used in the example 18 about each DNA.
[0066] Consequently, the amount of DNA was able to check significant absorption about the thing of 10pg(s), 1pg, and 100fg as compared with the background. Here, the amount of DNA stopped at absorption of background extent being shown about the thing of 10fg(s).
[0067] Example 21 (detection using a KK01 share probe (1))
DNA was prepared from nine sorts of each strain like the example 18. Detection which used the hybridization method like the example 7 was performed except using probe:5'GGACGGTGTGGAAGTGTCGCCGATGCTGGTGACCGGTGATA-Biotin3' of the following prepared in the example 17 about each DNA. Consequently, the very strong electropositive reaction has been checked only about what carried out the blot of the KK 01-share DNA. Here, in eight sorts of other strain, the electropositive reaction was undetectable.
[0068] Example 22 (detection using a KK01 share probe (2))
DNA was prepared from nine sorts of each strain like the example 18. Detection which used the hybridization method like the example 8 was performed except using probe:5'GGACGGTGTGGAAGTGTCGCCGATGCTGGTGACCGGTGATA-Biotin3' of the following prepared in the example 17 about each DNA. Consequently, although fluorescence was accepted in the fungus body about one share of JM, in other strain, fluorescence was unobservable.
[0069] Example 23 (preparation of the primer for TL1 shares)
Array number: Instead of 9 and 10, the oligonucleotide was compounded like the example 2 except using the following arrays.
5'AGCGTGAAGCTGCGCCTGATC3' (array number: 18)
5'ATGTGGGTGCCGGGGATGC3' (array number: 19)
Example 24 (preparation of the probe for TL1 shares)
Array number: The oligonucleotide was compounded like the example 3 except using the following arrays instead of 11.
5'TCCGAACTCGTTCTATGCAGCGGGCGA3' (array number: 20)
Example 25 (evaluation of the singularity of the primer for one share of TL)
As sample strain One share of P.sp.TL, one share of JM, P.cepacia One share of KKO, P. putida BH stock, P.aeruginosa IFO 3080 shares, P. fluorescens IFO 14160 shares, Alcaligenes faecalis IFO 14479 shares, Xanthomonasmaltophilia 14161 shares of IFO(s), Enterbacter cloacae 13535 shares of IFO(s), E.coli Ten sorts of bacteria of 109 shares of JM are used. As a primer 5'-Biotin-AGCGTGAAGCTGCGCCTGATC 3'5 -- magnification and its detection of the nucleic acid by PCR

were performed like the example 4 except having used '-DNP-ATGTGGGTGCCGGGGATGC3'. Consequently, only in one share of TL, one clear band has been checked to the die length of about 450 base pairs expected. Here, in nine sorts of other strain, any magnification nucleic acids were not detected at all.
[0070] Example 26 (detection using the primer of one share of TL (1))
DNA was prepared from ten sorts of each strain like the example 25. Magnification of the nucleic acid by PCR and the amplified nucleic-acid fragment were detected like the example 5 except using the primer used in the example 25 about each DNA.
[0071] Consequently, only in one share of TL, significant absorption was able to be checked as compared with the background. Here, nine sorts of other strain stopped at absorption of background extent being shown.
[0072] Example 27 (detection using the primer of one share of TL (2))
DNA was prepared from one share of TL like the example 25. Magnification of the nucleic acid by PCR and the amplified nucleic-acid fragment were detected like the example 6 except using the primer used in the example 25 about each DNA.
[0073] Consequently, the amount of DNA was able to check significant absorption about the thing of 10pg(s), 1pg, and 100fg as compared with the background. Here, the amount of DNA stopped at absorption of background extent being shown about the thing of 10fg(s).
[0074] Example 28 (detection using the probe of one share of TL (1))
DNA was prepared from ten sorts of each strain like the example 25. probe:5' of the following prepared in the example 24 about each DNA -- TCCGAACTCGTTCTATGCAGCGGGCGA3 -- detection which used the hybridization method like the example 7 was performed except using '-Biotin3'. Consequently, the very strong electropositive reaction has been checked only about what carried out the blot of the 1 share of TLDNA. Here, in eight sorts of other strain, the electropositive reaction was undetectable.
[0075] Example 29 (detection using the probe of one share of TL (2))
DNA was prepared from ten sorts of each strain like the example 25. probe:5' of the following prepared in the example 24 about each DNA -- TCCGAACTCGTTCTATGCAGCGGGCGA3 -- detection which used the hybridization method like the example 8 was performed except using '-Biotin3'. Consequently, although fluorescence was accepted in the fungus body about one share of TL, in other strain, fluorescence was unobservable.
[0076] Example 30 (preparation of the primer for TL2 shares)
Array number: Instead of 9 and 10, the oligonucleotide was compounded like the example 2 except using the following arrays.
5'GACGTGCAGAACCGCATCGTC3' (array number: 21)
5'TTGATGTACTCGACAAAACC3' (array number: 22)
Example 31 (preparation of the probe for TL2 shares)
Array number: The oligonucleotide was compounded like the example 3 except using the following arrays instead of 11.
5'AAGATCATCGGCGCCACCGACAAGCGCGGCACCGA3' (array number: 23)
Example 32 (evaluation of the singularity of the primer for two shares of TL)
As sample strain Alcaligenes Two shares of sp.TL, one share of JM, P.cepacia One share of KKO, P. putida BH stock, P.aeruginosa IFO 3080 shares, P. fluorescens IFO 14160 shares, Alcaligenes faecalis IFO 14479 shares, Xanthomonas maltophilia 14161 shares of IFO(s), Enterbacter cloacae 13535 shares of IFO(s), E.coli Ten sorts of bacteria of 109 shares of JM are used. As a primer 5'-Biotin-GACGTGCAGAACCGCATCGTC 3'5 -- magnification and its detection of the nucleic acid by PCR were performed like the example 4 except having used '-DNP-TTGATGTACTCGACAAAAC C3'. Consequently, only in two shares of TL, one clear band has been checked to the die length of about 300 base pairs expected. Here, in nine sorts of other strain, any magnification nucleic acids were not detected at all.
[0077] Example 33 (detection using the primer of two shares of TL (1))
DNA was prepared from ten sorts of each strain like the example 32. Magnification of the nucleic acid by PCR and the amplified nucleic-acid fragment were detected like the example 5 except using the primer used in the example 32 about each DNA.

[0078] Consequently, only in two shares of TL, significant absorption was able to be checked as compared with the background. Here, nine sorts of other strain stopped at absorption of background extent being shown.

[0079] Example 34 (detection using the primer of two shares of TL (2))

DNA was prepared from two shares of TL like the example 32. Magnification of the nucleic acid by PCR and the amplified nucleic-acid fragment were detected like the example 6 except using the primer used in the example 32 about each DNA.

[0080] Consequently, the amount of DNA was able to check significant absorption about the thing of 10pg(s), 1pg, and 100fg as compared with the background. Here, the amount of DNA stopped at absorption of background extent being shown about the thing of 10fg(s).

[0081] Example 35 (detection using the probe of two shares of TL (1))

DNA was prepared from ten sorts of each strain like the example 32. Detection which used the hybridization method like the example 7 was performed except using probe:5'AAGATCATCGGCGCCACCGACAAGCGCGGCACCGA-Biotin3' of the following prepared in the example 31 about each DNA. Consequently, the very strong electropositive reaction has been checked only about what carried out the blot of the 2 shares of TLDNA. Here, in nine sorts of other strain, the electropositive reaction was undetectable.

[0082] Example 36 (detection using the probe of two shares of TL (2))

DNA was prepared from ten sorts of each strain like the example 32. Detection which used the hybridization method like the example 8 was performed except using probe:5'AAGATCATCGGCGCCACCGACAAGCGCGGCACCGA-Biotin3' of the following prepared in the example 31 about each DNA. Consequently, although fluorescence was accepted in the fungus body about two shares of TL, in other strain, fluorescence was unobservable.

[0083] Example 37 (KB preparation of the primer for two shares)

Array number: Instead of 9 and 10, the oligonucleotide was compounded like the example 2 except using the following arrays.

5'TCCGTCTGGCTCAAGCTCACCGTC3' (array number: 24)

5'GCGCGAGCCGTTGGCATGGAAGG3' (array number: 25) example 38 (KB preparation of the probe for two shares)

Array number: The oligonucleotide was compounded like the example 3 except using the following arrays instead of 11.

5'TGCGGAAACCTATGGCGGCATTCCCGGCACCGAAA3' (array number: 26)

Example 39 (KB evaluation of the singularity of the primer for two shares)

As sample strain, it is P.alcaligenes. Two shares of KB(s), one share of JM, P. cepacia One share of KKO, P.putida BH stock, P. aeruginosa IFO 3080 shares, P.fluorescens IFO 14160 shares, Alcaligenes faecalis IFO 14479 shares, Xanthomonas maltophilia 14161 shares of IFO(s), Enterbacter cloacae 13535 shares of IFO(s), E.coli Ten sorts of bacteria of 109 shares of JM are used. As a primer 5'-Biotin-TCCGTCTGGCTCAAGCTCACCGTC 3'5 -- magnification and its detection of the nucleic acid by PCR were performed like the example 4 except having used '-DNP-GCGCGAGCCGTTGGCATGG AAGG3'. Consequently, only in two shares of KB(s), one clear band has been checked to the die length of about 390 base pairs expected. Here, in nine sorts of other strain, any magnification nucleic acids were not detected at all.

[0084] Example 40 (detection using a KB2 share primer (1))

DNA was prepared from ten sorts of each strain like the example 39. Magnification of the nucleic acid by PCR and the amplified nucleic-acid fragment were detected like the example 5 except using the primer used in the example 39 about each DNA.

[0085] Consequently, only in two shares of KB(s), significant absorption was able to be checked as compared with the background. Here, nine sorts of other strain stopped at absorption of background extent being shown.

[0086] Example 41 (detection using a KB2 share primer (2))

DNA was prepared from two shares of KB(s) like the example 39. Magnification of the nucleic acid by PCR and the amplified nucleic-acid fragment were detected like the example 6 except using the primer used in the example 39 about each DNA.

[0087] Consequently, the amount of DNA was able to check significant absorption about the thing of

10pg(s), 1pg, and 100fg as compared with the background. Here, the amount of DNA stopped at absorption of background extent being shown about the thing of 10fg(s).
[0088] Example 42 (detection using a KB2 share probe (1))
DNA was prepared from ten sorts of each strain like the example 39. Detection which used the hybridization method like the example 7 was performed except using probe:5'TGCGGAAACCTATGGCGGCATTCCCGGCACCGAAA-Biotin3' of the following prepared in the example 38 about each DNA. Consequently, the very strong electropositive reaction has been checked only about what carried out the blot of the 2 shares of KB(s)DNA. Here, in nine sorts of other strain, the electropositive reaction was undetectable.
[0089] Example 43 (detection using a KB2 share probe (2))
DNA was prepared from ten sorts of each strain like the example 39. Detection which used the hybridization method like the example 8 was performed except using probe:5'TGCGGAAACCTATGGCGGCATTCCCGGCACCGAAA-Biotin3' of the following prepared in the example 38 about each DNA. Consequently, although fluorescence was accepted in the fungus body about two shares of KB(s), in other strain, fluorescence was unobservable.
[0090]
[Effect of the Invention] Since the characteristic field in the gyrB gene which such strain has is used for detection of specific strain useful to purification of the contamination soil as a candidate for detection in this invention, it becomes possible to perform alternative and specific detection of such strain easily. By using the probe or primer chosen based on the information acquired from the base sequence of the gyrB gene of each strain offered by this invention, it becomes possible to offer the detection approach of strain of having excelled in respect of sensibility, singularity, simplicity, and quick nature, and a great contribution can be made in fields, such as environmental purification.
[0091]
[Layout Table]
array number: -- die-length [of one array]: -- mold [of 1092 arrays]: -- number [of nucleic-acid chains]: -- double strand topology: -- class [of straight chain-like array]: -- chromosome DNA origin: -- Vibrio One share (FERM P-14643) of sp.KB
description [of an array]: -- gyrB gene sequence: -- GACGGCACCG GCCTGCACCA-CCTTGTCTTC GAGGTGCTGG-ACAACTCCAT CGACGAAGCG 60CTGGCCGGCT CCGAGATCCA-GGTCACCATC CACAGCGACA-ACTCGATCTC CATCGTCGAC 120AACGGCCGCG GCATCCCGCC-CAAGTTCGAC GACAAGCACG-AACCCAAGCG CAGCGCGGCC 180GAAATCGCCA TGACCGAGCT-GCACGCCGGC TTCAACCAGA-ACAGCTACAA GGTGTCTGGC 240GGCCTGCACG GTGTGGGCGT GTCCTGCGTG AACGCCCTGT GGCTGCGCCT GACCGTGCGC 300CGCGACGGCC AGGTCCACCT GATCGAATTC GCCAAGGGCG ACGTACAGAA CGTCGAGACC 360GTGACCGGCC CCGACGGCCA GCCCGTTGAA GTCTCGCCGA TGAAGATCAT CGGCGCCACC 420CGCGGCACTG AAGTCCACTT CCTGGCCGAC GAAGAGATCT TCACCAACGT CGAGTTCCAC 480TACGAGATCC AGCGCATCCG CGAGCTCTCG TTCCTGAACA ACGGCGTGCA CATCAAGCTG 540GTCGACCAGC GCACCGGCAA AGACTTTGCC TTCTCCGGCG GCGTGAAGGG CTTTGTCGAG 600TACATCAACC GCTCCAAGAG CGTGCTGCAC ATCTTCTACG CCAACACCGA AAAAGACGGT 660ATCGCCGTGG AAGTGGCCAT GCAGTGGAAC GACGGCTACA AGGTGCTCTG CTTCACCAAC 720AACATCCCGC AGCGGGATGG CGGCACCCAC CTGACCGGCC TGCGCGCCGC CCGCGTCATC 780AACAAGTACA TTGAAGAGAA CGAAGTCGCC AAGAAAGCCA AGGTGGAAAC CACCGGCGAC 840CGCGAAGGCC TGTCCTGCGT GCTCTCCGTC AAGGTGCCCG AGCCCAAGTT CAGCTCGCAG 900ACCAAGGACA TCTCTTCCGA AGTGCGCCTG CCCGTGGAAG AACTCGTCGG CAAGGCCCTG 960AACGACTTCC TGCTGGAAAC CGACGCCAAG ATCATCTGCG GCAAGATCGT AGACGCCGCC 1020 CGCGCCCGCG AAGCCGCCCG CAAGGCCCGC ACGCGCCGCA AGGGCCTGAT GGACGGCATG 1080GGCCTGCCCG GC 1092 array number: -- die-length [of two arrays]: -- mold [of 1021 arrays]: -- number [of nucleic-acid chains]: -- double strand topology: -- class [of straight chain-like array]: -- chromosome DNA origin: -- one share (FERM BP-5352) of JM
description [of an array]: -- gyrB gene sequence: -- GGTTCAGGCT TACACCATAT-

GGTGTTTGAG GTGGTGGATA-ATGCGATCGA TGAGGCGCTT 60GCAGGTCACT AAATTTTGGT-GACGATTCAC GAAGATGAAT-CTGTAAGTGT ATCGGACAAT 120GGGCGCGGTA TTCCGACCGA-CCCTGAAGAA GGGGTTTCTG-CCGCAGAAGT GATTTTAACC 180ATTCTGCACG CAGGTGGTAA-ATTTGATGAC TACAAGGTTT-CAGGTGGTCT ACATGGTGTA 240GGCGTATCGG TTGTGAATGC CTTGTCACGT AAGTTGGAAC TTCATCGTGC AGGTCATATT 300CATCAACAAG AATATAAACA CGGTGATCCG GTTTACCCAC TGACTGTTGT TACGGATACG 360ACAGGAACAA CTGTCCGTTT TTGGCCAAGT GCTGAAACTT TTAGCCAAAC CATTTTCAAT 420ATTCTAGCGC GTCGTTTACG TGAATTGTCT TTCTTAAACG CGGGCGTGCG CATTGTTTTG 480CGTGACGAGC ATGCAGAACA TGTGTTTGAC TACGAAGGCG GTCTGTCTGA GTTTGTGAAA 540TATATTAACC AAGGCAAAAC GAACGACATT TTCCATTTCA CCGTACAGGC TGAAAATGGC 600ATTGGTGTTG AAGTGGCGTT GCAGTGGAAT TATCAAGAAA ACGTACGTTG CTTTACCAAC 660AACATTCCAC AAAAAGATGG TGGAACGCAT TTAGCGGGTT CGGCATTAAC GCGTGGTCTA 720AACAGCTACA TGGAAAACGA AAACTTACTC AAAAAAGAGA AAGTAGCGGT TGATGATGCA 780CGTGAAGGTT TAACTGCGAT TGTTTCAGTG AAAGTGCCTG ATCCGAAGTT CTCTTCTCAG 840GAAAAGTTGG TTTCAAGTGA AGTGAAACCT GCGGTTGAGC AGGCAATGAA TAAATCATTC 900TCTGAATATT AAAACCCGCA AGCTGCAAAA TCAATTGCAG GCAAAATCAT TGATGCGGCA 960CGTGCTCGTG ATGCAGCACG GCGTGAAATG ACGCGTCGTA AAGTGCGTT AGACATTGCA 1020G
1021 array number: -- die-length [of three arrays]: -- mold [of 924 arrays]: -- number [of nucleic-acid chains]: -- double strand topology: -- class [of straight chain-like array]: -- the chromosome DNA origin :P seudomonas cepacia KK01 share (FERM BP-4235)
description [of an array]: -- gyrB gene sequence: -- GACGGCACCG GTCTGCACCA-CCTCGTGTTC GAGGTGCTCG-ACAACTCGAT CGACGAAGCG 60CTGGCCGGGT ACTGCAACGA-CATCCACGTG ACGATTCACG-CCGACAACTC GATTTCCGTG 120ACCGACAACG GCCGCGGGAT-TCCGACCGAC GTGAAGATGA-ACGACAAGCA CGAGCCGAAG 180CGCAGCGCCG CCGAGATCGT-GATGACCGAG CTGCATGCCG-GCGGCAAGTT CGACCAGAAC 240AGCTACAAGG TGTCCGGCGG CCTGCACGGC GTGGGTGTGT CGTGCGTGAA CGCGCTGTCG 300AGCTGGCTGC GCCTCACCGT GCGCCGCGGC GGCAAGAAGC GTTTCATGGA GTTCCATCGC 360GGCATCGCGC AGGATCGCGT GCTCGAGCAG GTGGACGGTG TGGAAGTGTC GCCGATGCTG 420GTGACCGGTG ATACCGAGAA CCGCGGCACC GAAGTGCACT TCATGGCCGA TCCGACCATT 480TTCGGCACGG TCGAGTATCA CTACGACATC CTCGCCAAGC GGATGCGTGA GCTCTCGTTC 540CTGAACAACG GCGTGCGGAT TCGTCTCACG GACCTGCGCT CGGGCAAGGA AGACGATTTC 600GCATTCGCCG GCGGCGTGAA GGGCTTCGTC GAGTACATCA ACAAGACGAA GACCAACCTG 660CACCCGACGA TTTTCTTCGC CACCGGCGAG AAGGATAACG TGGGCGTCGA AGTCGCGATG 720CAGTGGAACG ACAGCTACAA CGAAAACGTG CTGTGCTTCA CGAACAACAT TCCGCAGCGC 780GACGGCGGCA CGCACCTGAC CGGCCTGCGG GCCGCGATGA CGCGCGTCAT CAACAAGTAC 840 ATCACCGACA ACGAAATCGC GAAGAAGGCC AAGGTCGAGA CGACCGGCGA CGACATGCGC 900 GAAGGGCTGT CGTGCGTGCT GTCG
924 array number: -- die-length [of four arrays]: -- mold [of 1120 arrays]: -- number [of nucleic-acid chains]: -- double strand topology: -- class [of straight chain-like array]: -- 1 share (FERM P-14726) of chromosome DNA origin-sp.TL
 seudomonas description [of an array]: -- gyrB gene sequence: -- GACGGCACGG GCCTGCACCA-CCTGGTGTTC GAAGTGGTGG-ACAACTCCAT CGACGAAGCG 60CTCGCGGGCC ACGACATCGT-CGTCACGATC CACTCCGACA-ACTCGATCTC CGTCACCGAC 120AACGGCCGCG GCATCCCGAC-GAAGATGGAC GACAAGCACG-AGCCCAAGCG CTCAGCCGCT 180GAAATCGCGC TCACCGAACT-GCACGCCGGC TTCAACCAGA-ACAGCTACAA GGTCTCGGGC 240GGCCTGCACG GCGTGGGCGT CTCGTGCGTG AACGCGCTGA AGCTGCGCCT GATCGTGCGC 300CGCGAAGGCA AGATCCACGA ACTCGAATTC AGCCGCGGCT TCGTGCAGAA GCTCGAAACC 360GTGAACGGCT TCGAGGTCTC GCCCATGAAG GTCATCGGCG ACACCGACAA GCGCGGCACC 420CACTTCCTGC CCGACACGGA

AATCTTCAAG GAAAACAACG ATTTCCACTA CGAAATCCTG 480AGCAAGCGCC AGTTGAGCTT CCTGAACAAC GGCGTGCGCA TCCGCCTGCT CGACGAGCGC 540ACCGGCAAGG AAGACGACTT CGCCGGCGGC GTGCGCGGCT TCGTGGAGTT CATCAACAAG 600GGCAAGACCG TCCTGCATCC GAACTCGTTC GCGGGCGAGA AGCCGGCCGA CACCTACGGC 660GGCATCCCCG GCACCCACAT CGGCGTCGAA GTGGCGATGC ACAGCGGCTA CAACGAGCAG 720GTCCTCTGTT TCACCAACAA CATCCCCCAG CGTGACGGCG GCACCCACCT CCTGCGCGCC 780GCGATGACCC GCGTCATCAA CAAGTACATC GAAGAGAACG AGTTCGCGAA AAAGGCGAAG 840GTCACCGGCG ACGACATGCG CGAAGGCTTG TGCTGCGTGC TGAGCGTGAA GGTGCCCGAG 900CCCAAGTTTT AGACCAAGGA CAAGCTGGTG TCCAGCGAAG TGCGCGCACC GGTGGAAGAC 960ATCGTCGGTC GCCTGCTCAC CCTGCAGGAG CGTCCGAACG ACGCCAAGAT CATCTGCGGC 1020AAGATCGTCG AGGCCGCCCG CGCCCGCGAA CGCAAGGCCC GCGAAATGAC GCGCCGCAAG 1080 GGCGTGCTCG ACGGCATGGG CCTGCCCGGC AAGCTGGCCG 1120 array number: -- die-length [of five arrays]: -- mold [of 867 arrays]: -- number [of nucleic-acid chains]: -- double strand topology: -- class [of straight chain-like array]: -- chromosome DNA origin: -- 2 shares (FERM P-14642) of Alcaligenes sp.TL
 description [of an array]: -- gyrB gene sequence: -- GACGGCACCG GCCTGCACCA-CCTTGTCTTC GAGGTGCTGG-ACAACTCCAT CGACGAAGCG 60CTGGCCGGCT CCGAGATCCA-GGTCACCATC CACAGCGACA-ACTCGATCTC CATCGTCGAC 120AACGGCCGCG GCATCCCGCC-CAAGTTCGAC GACAAGCACG-AACCCAAGCG CAGCGCGGCC 180GAAATCGCCA TGACCGAGCT-GCACGCCGGC TTCAACCAGA-ACAGCTACAA GGTGTCTGGC 240GGCCTGCACG GCGTGGGCGT GTCCTGCGTG AACGCCCTGT GGCTGCGCCT GACCGTGCGC 300CGCGACGGCC AGGTCCACCT GATCGAATTC GCCAAGGGCG ACGTGCAGAA CGTCGAGACC 360GTGACCGGCC CCGACGGCCA GCCCGTTGAA GTCTCGCCGA TGAAGATCAT CGGCGCCACC 420CGCGGCACCG AAGTCCACTT CCTGGCCGAC GAAGAGATCT TCACCAACGT CGAGTTCCAC 480TACGAGATCC AGCGCATCCG CGAGCTCTCG TTCCTGAACA ACGGCGTGCA CATCAAGCTG 540GTCGACCAGC GCACCGGCAA AGACTTTGCC TTCTCCGGCG GCGTGAAGGG TTTTGTCGAG 600TACATCAACC GCTCCAAGAG CGTGCTGCAC ATCTTCTACG CCAACACCGA AAAAGACGGT 660ATCGCCGTGG AAGTGGCCAT GCAGTGGAAC GACGGCTACA AGGTGCTCTG CTTCACCAAC 720AACATCCCGC AGCGCGATGG CGGCACCCAC CTGACCGGCC TGCGCGCCGC CCGCGTCATC 780 AACAAGTACA TTGAAGAGAA CGAAGTCGCC AAGAAAGCCA AGGTGGAAAC CACCGGCGAC 840 CGCGAAGGCC TGTCCTGCGT GCTCTCC 867 array number: -- die-length [of six arrays]: -- mold [of 886 arrays]: -- number [of nucleic-acid chains]: -- double strand topology: -- class [of straight chain-like array]: -- the chromosome DNA origin :P seudomonas alcaligenes Two shares (FERM BP-5354) of KB(s) description [of an array]: -- gyrB gene sequence: -- GACGGCACCG GTCTGCACCA-CCTGGTCTTC GAGGTCGTGG-ACAACTCCAT CGACGAAGCG 60CTGGCCGGCT ACGACATTCT-GGTCACCATC CATGCCGACG-GCTCGCTGTC CGTCATCGAC 120AACGGCCGCG GCATTCCCAC-GAAGATGGAC GACAAGCACG-AGCCCAAGCG CTCGGCTGCC 180GAAATCGCGC TGACCGAGCT-GCACGCCGGC TTCAACCAGA-ACAGCTACAA GGTCTCGGGC 240GGTCTGCACG GCGTGGGCGT GTCCTGCGTG AACGCGCTGT GGCTCAAGCT CACCGTCAGC 300CGCGACGGCC GACGCCACGA AATCGACTTC TCGCGCGGCT TTGTGCAAAA GATCGAAGTC 360GTGGACGGCG CCGAAGTCTC GCCCATGCGC GTGGTCGGCG CCAGCGACAA GCGCGGCACC 420CACTTTCTGC CCGACCAGGA GATCTTCAAG GAGAACTTCG AGTTCCGCTA CGAGATTCTG 480GCCAAGCGCC AGCTGTCCTT CCTGAACAAC GGCGTGCGCA TCCGCTTGAA GGACGAGCGC 540GACGGCAAGG AAGACGACTT CGCCGGCGGC GTCAAGGGCT TTGTGCAGTT CATCAACGGC 600ACCAAGAAGG TTTTGCACCC CACCACCTTC AACGGCTCGC GCCCTGCGGA AACCTATGGC 660GGCATTCCCG GCACCGAAAT CGGTGTCGAA GTGTCCATGC ACGACAGCTA TGCCGAGCAG 720GTGCTGTGCT TCACCAACAA CATTCCCCAG CGTGACGGCG GCACCCATCT CCTGCGCGCC 780GCCATGACCC GCGTGATCGG CAAATACATC GCCGACAACG

AAATGGCCAA GAAGGCCAAG 840GTCTCCGGCG ACGACATGCG CGAAGGCCTG TGCGCCGTTC TCAGCG886 array number: -- die-length [of seven arrays]: -- mold [of 41 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array description [of a synthetic DNA array]: -- common primer array for gyrB gene detection: -- CAGGAAACAG CTATGACCAA RMGICCNGGI ATGTAYATHG G 41 array number: -- die-length [of eight arrays]: -- mold [of 38 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array description [of a synthetic DNA array]: -- common primer array for gyrB gene detection: -- TGTAAAACGA CGGCCAGTAA YTTNGGNTCN GGNACYTT 38 array number: -- die-length [of nine arrays]: -- mold [of 23 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array description [of a synthetic DNA array]: -- primer array [in a gyrB gene] for base sequence detection: -- CGCCAAGGGC GACGTACAGA ACC 23 array number: -- die-length [of ten arrays]: -- mold [of 21 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array description [of a synthetic DNA array]: -- primer array [in a gyrB gene] for base sequence detection: -- GTGCAGCACG CTCTTGGAGC G 21 array number: -- die-length [of 11 arrays]: -- mold [of 31 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array description [of a synthetic DNA array]: -- probe array [in a gyrB gene] for base sequence detection: -- GCGCCACCGA CAAGCGCGGC ACTGAAGTCC A 31 array number: -- die-length [of 12 arrays]: -- mold [of 23 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array description [of a synthetic DNA array]: -- primer array [in a gyrB gene] for base sequence detection: -- GAATGCCTTG TCACGTAAGT TGG 23 array number: -- die-length [of 13 arrays]: -- mold [of 23 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array description [of a synthetic DNA array]: -- primer array [in a gyrB gene] for base sequence detection: -- CACTGAAACA ATCGCAGTTA AAC 23 array number: -- die-length [of 14 arrays]: -- mold [of 36 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array description [of a synthetic DNA array]: -- probe array [in a gyrB gene] for base sequence detection: -- CGTATCGGTT GTGAATGCCT TGTCACGTAA GTTGGA 36 array number: -- die-length [of 15 arrays]: -- mold [of 25 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array description [of a synthetic DNA array]: -- primer array [in a gyrB gene] for base sequence detection: -- CGTGCTCGAG CAGGTGGACG GTGTG 25 array number: -- die-length [of 16 arrays]: -- mold [of 30 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array description [of a synthetic DNA array]: -- primer array [in a gyrB gene] for base sequence detection: -- TTATCCTTCT CGCCGGTGGC GAAGAAAATC 30 array number: -- die-length [of 17 arrays]: -- mold [of 41 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array description [of a synthetic DNA array]: -- probe array [in a gyrB gene] for base sequence detection: -- GGACGGTGTG GAAGTGTCGC CGATGCTGGT GACCGGTGAT A 41 array number: -- die-length [of 18 arrays]: -- mold [of 21 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array description [of a synthetic DNA array]: -- primer array [in a gyrB gene] for base sequence detection: -- AGCGTGAAGC TGCGCCTGAT C 21 array number: -- die-length [of 19 arrays]: -- mold [of 19 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array description [of a synthetic DNA array]: -- primer array [in a gyrB gene] for base sequence detection: -- ATGTGGGTGC CGGGGATGC 19 array number: -- die-length [of 20 arrays]: -- mold [of 27 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array description [of a synthetic DNA array]: -- probe array [in a gyrB gene] for base sequence detection: -- TCCGAACTCG TTCTATGCAG CGGGCGA 27 array number: -- die-length [of 21 arrays]: -- mold [of an array]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array description [of a synthetic DNA array]: -- primer array [in a gyrB gene] for base sequence detection: --

GACGTGCAGA ACCGCATCGT C 21 array number: -- die-length [of 22 arrays]: -- mold [of 20 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array description [of a synthetic DNA array]: -- primer array [in a gyrB gene] for base sequence detection: -- TTGATGTACT CGACAAAACC array number: -- die-length [of 23 arrays]: -- mold [of 35 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array description [of a synthetic DNA array]: -- probe array [in a gyrB gene] for base sequence detection: -- AAGATCATCG GCGCCACCGA CAAGCGCGGC ACCGA 35 array number: -- die-length [of 24 arrays]: -- mold [of 24 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array description [of a synthetic DNA array]: -- primer array [in a gyrB gene] for base sequence detection: -- TCCGTCTGGC TCAAGCTCAC CGTC 24 array number: -- die-length [of 25 arrays]: -- mold [of 23 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array description [of a synthetic DNA array]: -- Plummer array [in a gyrB gene] for base sequence detection: -- GCGCGAGCCG TTGGCATGGA AGG 23 array number: -- die-length [of 26 arrays]: -- mold [of 35 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array description [of a synthetic DNA array]: -- probe array [in a gyrB gene] for base sequence detection: -- TGCGGAAACC TATGGCGGCA TTCCCGGCAC CGAAA 35

[Translation done.]